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FLEAS OF BLACK-FOOTED FERRETS AND THEIR POTENTIAL ROLE IN THE MOVEMENT OF PLAGUE

Erica L. Mize,[1,2,4] Shaun M. Grassel,[3] and Hugh B. Britten[1]

[1] Department of Biology, University of South Dakota, 414 East Clark St., Vermillion, South Dakota 57069, USA
[2] Midwest Fisheries Center, US Fish and Wildlife Service, 555 Lester Ave., Onalaska, Wisconsin 54650, USA
[3] Department of Wildlife, Fish, and Recreation, Lower Brule Sioux Tribe, 22695 Little Bend Rd. PO Box 246, Lower Brule, South Dakota 57548, USA
[4] Corresponding author (email: erica_mize@fws.gov)

ABSTRACT: Sylvatic plague is one of the major impediments to the recovery of the black-footed ferret (*Mustela nigripes*) because it decimates their primary prey species, prairie dogs (*Cynomys* spp.), and directly causes mortality in ferrets. Fleas are the primary vector of *Yersinia pestis*, the causative agent of sylvatic plague. The goal of this research was to better understand the flea fauna of ferrets and the factors that might influence flea abundance on ferrets. Fleas from ferrets were tested for *Y. pestis* in a post hoc assessment to investigate the plausibility that some ferrets could act as incidental transporter hosts of fleas infected with *Y. pestis*. Fleas were collected from ferrets captured on the Lower Brule Indian Reservation in central South Dakota from 2009 to 2012. A total of 528 fleas collected from 67 individual ferrets were identified and tested for the presence of *Y. pestis* with a nested PCR assay. The predominant flea recovered from ferrets was *Oropsylla hirsuta*, a species that comprises 70–100% of the fleas recovered from prairie dogs and their burrows in the study area. *Yersinia pestis* was detected at low levels in fleas collected from ferrets with prevalence ranging from 0 to 2.9%; male ferrets harbored significantly more fleas than female ferrets. Six of 67 ferrets vaccinated against plague carried fleas that tested positive for *Y. pestis*, which suggests ferrets vaccinated against plague could inadvertently act as incidental transporter hosts of *Y. pestis*–positive fleas.

Key words: Black-footed ferret, *Oropsylla hirsuta*, plague, *Yersinia pestis*

INTRODUCTION

The black-footed ferret (*Mustela nigripes;* hereafter ferret) was historically distributed across western North America, driven to apparent extinction, and rediscovered (Thorne and Williams 1988). The ferret is currently listed on the US Endangered Species List (US Fish and Wildlife Service 1967, 2012). A captive breeding program has been successful in re-establishing some wild populations, yet disease and availability of prey remain significant threats to its survival (US Fish and Wildlife Service 2012). Ferrets are obligate predators of prairie dogs (*Cynomys* spp.), and both are highly susceptible to sylvatic plague (Gage and Kosoy 2005; Godbey et al. 2006; Matchett et al. 2010), which is caused by the bacterium *Yersinia pestis*. Plague epizootics can reduce the number of prairie dogs within colonies by up to 100%, causing localized extirpation of both prairie dogs and ferrets (Gage and Kosoy 2005, 2006). Because plague has impacted nearly all ferret reintroduction sites (Godbey et al. 2006), successful reintroduction and establishment of self-sustaining populations of ferrets will not be possible without a better understanding of plague dynamics and the effects of sylvatic plague on ferrets and prairie dogs.

Black-tailed prairie dogs (*Cynomys ludovicianus*) in South Dakota are parasitized primarily by two flea species, *Oropsylla hirsuta* and *Oropsylla tuberculata* (Brinkerhoff 2008; Mize and Britten 2016); both are capable of transmitting *Y. pestis* (Wilder et al. 2008a, b). However, population genetic studies of *O. hirsuta* suggest black-tailed prairie dogs are not the primary disperser of these fleas within and among their colonies (Jones and Britten 2010; Brinkerhoff et al. 2011). Plague exposure has been reported in many wildlife species including badgers (*Taxidea taxus*), coyotes (*Canis latrans*), raccoons (*Procyon lotor*), red fox (*Vulpes vulpes*), skunks (*Mephitis* spp., Salkeld and Stapp

2006; Brinkerhoff et al. 2009), and swift fox (*V. velox*; Salkeld et al. 2007). However, among these species, only coyotes have somewhat of an overlap in flea fauna (<5% *O. hirsuta*) with prairie dogs (Brinkerhoff 2008). Northern grasshopper mice (*Onychomys leucogaster*) can support high numbers of *O. hirsuta*, which could exacerbate plague outbreaks in prairie dog populations by increasing the spread of infected fleas among prairie dogs when their populations are high (Salkeld and Stapp 2009; Stapp et al. 2009; Salkeld et al. 2010). Recent research has indicated that *O. hirsuta* was the most abundant ectoparasite of ferrets in the Conata Basin, South Dakota, USA (Harris et al. 2014).

Our goal was to determine the occurrence and prevalence of flea species on ferrets in the study area. We also evaluated the influence of factors such as sex, age, colony, Julian date of collection, and insecticidal treatment on the abundance of fleas on ferrets. Fleas were also collected from prairie dogs and their burrows at the same site in 2009 and 2010 (Britten and Mize 2010). Because of their close association with prairie dogs, we questioned if ferrets might be capable of transporting infected fleas across and among colonies, thus potentially contributing to local plague epizootics. Fleas from ferrets were tested for *Y. pestis* in a post hoc assessment to investigate the plausibility that some ferrets could act as incidental transporter hosts of fleas infected with *Y. pestis*.

MATERIALS AND METHODS

We conducted our study on the Lower Brule Indian Reservation (LBIR) in central South Dakota, USA (43°59′N, 99°29′W). Ferrets were first reintroduced to colonies of black-tailed prairie dogs at this site in 2006 (US Fish and Wildlife Service 2012). We collected *Y. pestis*–positive fleas from burrows of prairie dogs in 2009 and 2010 (Britten and Mize 2010), and the first recorded plague epizootics occurred on the Reservation in 2011 and 2013. In 2010, there were an estimated 124 prairie dog colonies totaling 2,633 ha (range=0.5–443.1 ha, $\bar{x}$=21.2 ha) on LBIR. Our study focused on 12 colonies totaling 850 ha (range=4.3–443.1 ha, $\bar{x}$=70.8 ha). Deltamethrin (Bayer, Leverkusen, Germany), an insecticide used to control fleas (Seery et al. 2003; Biggins et al. 2010; Matchett et al. 2010), was applied to the prairie dog burrows of several colonies by site managers during our study to control plague (Fig. 1). The plague epizootic that occurred in 2011 reduced the number of colonies on the LBIR considerably, including some that were included in our study (Fig. 1).

Wild-born ferrets captured during night-time spotlight surveys were examined for fleas during fall 2009–12. Ferrets were captured using wire cage traps (91.5×10×10 cm) placed into the opening of prairie dog burrows occupied by ferrets. Each ferret was anesthetized using isoflurane in a top-opening induction chamber (41×21×27 cm) and systematically combed for fleas using 20 strokes with a standard flea comb (Gage 1999). Ferrets were combed over the induction chamber, which allowed anesthetized fleas to fall into the induction chamber where they remained anesthetized for several minutes. All fleas that fell into the induction chamber were collected for analysis. The white sides and bottom of the induction chamber aided in the detection and collection of fleas. Flea detection rates were not determined in the current study, and we are unaware of any flea detection rate studies for ferrets, but standardized methodology should have allowed for valid comparisons in our analyses (see Eads et al. 2013 for detection rates in prairie dogs using similar combing techniques). Sex and age class (juvenile or adult) of each ferret was recorded (Santymire et al. 2012). Prior to release at their capture sites, all ferrets were treated with fipronil (Merial, Duluth, Georgia, USA) to reduce flea populations and vaccinated with F1-V fusion protein vaccine for protection against sylvatic plague, with recaptures receiving a second dose (Rocke et al. 2004; Powell et al. 2005; Rocke et al. 2008). Therefore, on initial capture, fleas were collected from unvaccinated ferrets, but, on recapture, flea collections were from ferrets that had received at least one vaccination.

Individual fleas were stored in 70% ethanol and identified (Furman and Catts 1982; Lewis 2002). Whole genomic DNA was extracted from fleas using PrepGem (Zygem, Hamilton, New Zealand) extraction kits. The DNA extraction performed on fleas also extracted DNA from *Y. pestis* when present. The presence of *Y. pestis* was assessed using a nested PCR developed by Hinnebusch and Schwan (1993), later modified by Hanson et al. (2007) targeting the *pla* gene, which is specific to *Y. pestis* (Sodeinde and Goguen 1988).

We estimated the total number of fleas collected across all ferrets, prevalence of flea-infested ferrets (number of infested ferrets/total number of ferrets), prevalence of *Y. pestis*–positive fleas on infested ferrets (number of *Y. pestis*–positive fleas/total number of flea-infested

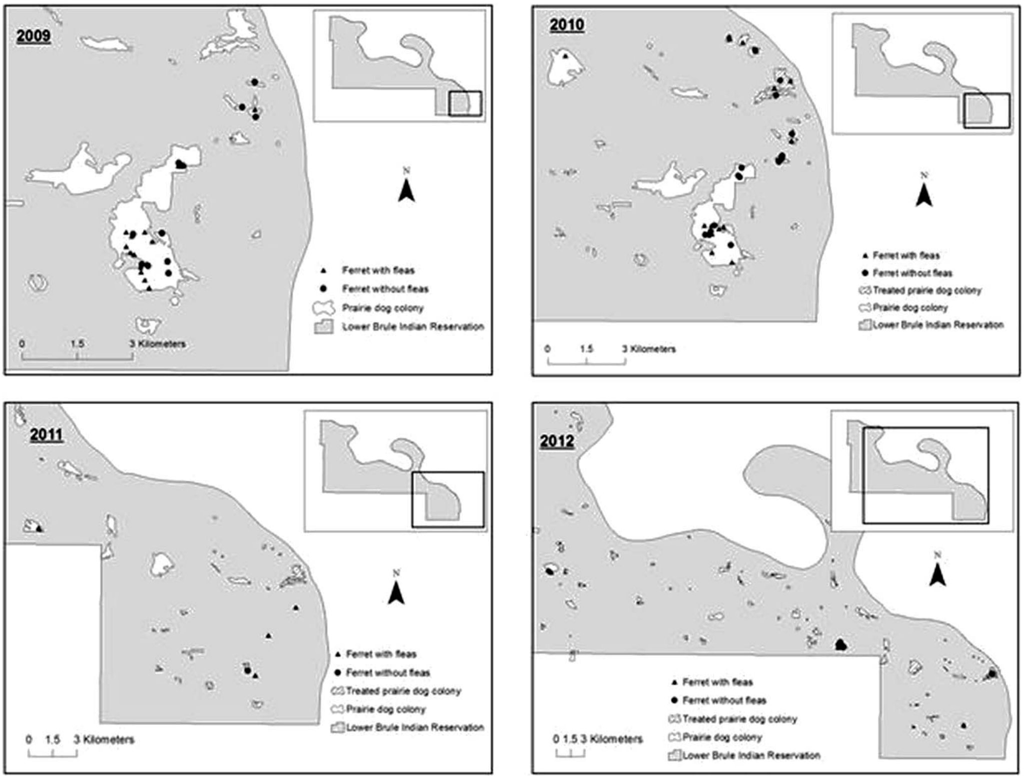

FIGURE 1. Locations of black-footed ferrets (*Mustela nigripes*) captured on colonies of black-tailed prairie dogs (*Cynomys ludovicianus*) on the Lower Brule Indian Reservation (South Dakota, USA), 2009–12. The map extents differ across years because the distribution of ferrets changed during the study due to natural and assisted dispersal.

ferret hosts), prevalence of *Y. pestis*–positive fleas collected from ferrets (number of *Y. pestis*–positive fleas/total number of fleas), and mean intensity of flea infestation per flea-infested ferret (number of fleas/number of infested ferrets), with appropriate 95% confidence intervals (Margolis et al. 1982) using Quantitative Parasitology v. 3.0 (Rózsa et al. 2000). Aggregation was examined for all fleas as well as fleas testing positive for *Y. pestis*. Degree of aggregation was estimated using a maximum-likelihood method for calculating k, the degree of aggregation of a population of organisms, where $k<1$ is considered an aggregated distribution (Rózsa et al. 2000; Wilson et al. 2002). The variance-to-mean ratio (σ^2:$\bar{x}$) of fleas collected from ferrets was also used as a measure of aggregation, with values greater than 1 representing a more aggregated distribution (Wilson et al. 2002). Both measures of aggregation were estimated using Quantitative Parasitology v. 3.0 (Rózsa et al. 2000).

We modeled factors that potentially influenced the abundance of *O. hirsuta* collected from ferrets with generalized linear models (GLMs) fitted with a negative binomial distribution (Shaw and Dobson 1998; Wilson et al. 2002) using program R v. 3.0.2 (R Development Core Team 2013) and the MASS package (Venables and Ripley 2002). We constructed 10 a priori candidate models representing multiple hypotheses and evaluated them using an information theoretic approach, with models ranked via Akaike's information criterion adjusted for small sample size (AIC_c; Burnham and Anderson 2002). We considered factors such as ferret sex and age, Julian date, year, the colony where individual ferrets were captured, and whether a colony had been treated with deltamethrin within the previous 6 mo prior to ferret capture. To confirm, we compared goodness-of-fit of our data to both negative binomial and Poisson distributions using a log likelihood test in the pscl package (Zuur et al. 2009). Our GLM analyses did not include fleas from recaptured ferrets because flea combing and fipronil treat-

TABLE 1. Total number of black-footed ferrets (*Mustela nigripes*) captured at Lower Brule Indian Reservation (South Dakota, USA) from 2009 to 2012 by age class and sex.

Year	Number			
	Adult female	Adult male	Kit female	Kit male
2009	4	4	7	11
2010	5	5	8	12
2011	2	1	3	2
2012	3	1	7	3
Total 2009–12	14	11	25	28

TABLE 3. Prevalence of *Yersinia pestis* in *Oropsylla hirsuta* collected from black-footed ferrets (*Mustela nigripes*) at Lower Brule Indian Reservation (South Dakota, USA) from 2009 to 2012.

Year	*Yersinia pestis* occurrence	
	No. positive	% Prevalence (95% CI)
2009	4	2 (1–6)
2010	0	0 (0–4)
2011	1	1 (0–6)
2012	5	3 (1–7)
Totals	10	2 (1–4)

ment likely affected the number of fleas on ferrets during subsequent captures.

RESULTS

A total of 528 fleas were collected from 67 unique wild-born ferrets, some of which were recaptured (30 recaptures; Table 1) from 2009 to 2012. Of the 528 fleas collected, 98.3% (n=519) were *O. hirsuta* (Table 2). Sample sizes of the other flea species collected were not large enough to include in analyses. The other nine fleas collected were *Epitedia wenmanni* (n=1), *Peromyscopsylla selenis* (n=1), unknown *Oropsylla* spp. (n=3), fleas from Family Ceratophyllidae (n=2), and unidentifiable fleas (n=2). All 10 fleas testing positive for the presence of *Y. pestis* were collected from male ferrets, but our power to detect significant differences in the number of *Y. pestis*–positive fleas between male and female ferrets was insufficient (power=11% using NQuery Advisor (Statsols, Boston, Massachusetts, USA), based on Fisher's exact test with an α=0.05 and a two-sided test).

The number of *Y. pestis*–positive fleas collected from individual flea-infested ferrets ranged from 0 to 5, and the annual prevalence of *Y. pestis*–positive fleas ranged from 0.0 to 2.9% (Table 3). Annual prevalence of ferrets carrying *Y. pestis*–positive fleas ranged from 0.0 to 22.2% (Table 4) with a mean intensity of infestation ranging from 0 to 2.5 *Y. pestis*–positive fleas per ferret carrying *Y. pestis*–positive fleas (Table 4).

Both the variance to mean ratio and aggregation coefficient k showed infestation by *O. hirsuta* on ferrets was highly aggregated across all years (Table 2). *Yersinia pestis*–positive fleas on ferrets also showed an aggregated distribution. However, the vari-

TABLE 2. Prevalence, intensity, and aggregation (k and σ^2:$\bar{x}$) of the flea *Oropsylla hirsuta* on black-footed ferret (*Mustela nigripes*) hosts at Lower Brule Indian Reservation (South Dakota, USA) from 2009 to 2012.

Year	No. fleas	No. ferret hosts	Percentage prevalence (95% CI)	Mean intensity (95% CI)[a]	k[b]	σ^2:$\bar{x}$[c]
2009	170	16	61.5 (42.2–78.8)	10.6 (6.3–16.3)	0.32	14.44
2010	84	16	53.3 (34.3–71.7)	5.3 (2.9–8.2)	0.33*	8.53
2011	93	6	75.0 (34.9–96.8)	15.5 (7.2–36.7)	0.50	23.10
2012	172	9	64.3 (35.1–87.2)	19.1 (8.7–32.4)	0.29	25.19
Totals	519	43	64.2 (51.5–75.5)	12.3 (8.6–16.7)	0.33	18.97

[a] Bootstrap confidence interval (BC_a) of Efron and Tibshirani (1993).

[b] Degree of aggregation, maximum-likelihood method for estimating k (Rózsa et al. 2000; Wilson et al. 2002).

[c] Variance to mean ratio σ^2:$\bar{x}$ where values >1 indicate an aggregated distribution (Rózsa et al. 2000; Wilson et al. 2002).

TABLE 4. Prevalence, intensity, and aggregation (variance to mean ratio $\sigma^2{:}\bar{x}$) of black-footed ferrets (*Mustela nigripes*) infested with *Yersinia pestis*–positive *Oropsylla hirsuta* at Lower Brule Indian Reservation (South Dakota, USA) from 2009 to 2012.

	Yersinia pestis occurrence			
Year	No. ferrets infected	Prevalence (95% CI)	Mean intensity (95% CI)[a]	Aggregation $\sigma^2{:}\bar{x}$[b]
2009	3	19 (4–46)	1.3 (1.0–1.7)	1.33
2010	0	0 (0–21)	0.0 (—)	—
2011	1	17 (0–64)	1.0 (—)	1.00
2012	2	22 (3–60)	2.5 (2.0–2.5)	2.30
Total	6	9 (3–19)	1.7 (1.2–2.2)	1.88

[a] Bootstrap confidence interval (BC_a) of Efron and Tibshirani (1993).

[b] Variance to mean ratio $\sigma^2{:}\bar{x}$ where values >1 indicate an aggregated distribution (Rózsa et al. 2000; Wilson et al. 2002).

ance-to-mean ratio was close to 1, indicating a low threshold of aggregation (Table 4). Aggregation could not be assessed for *Y. pestis*–positive fleas infesting ferrets collected in 2010 and 2011 because sample sizes were too low.

The top two candidate models, based on AIC_c scores, both included sex and year as covariates; treatment with deltamethrin was included in the second competing top model (Table 5). We selected the model that contained sex and year as the single best approximating model because it had the lowest ΔAIC_c score and the fewest variables (Table 5). We interpreted the other competing top model with $\Delta AIC_c<2$ as a nested version of the best model and considered the additional covariate for deltamethrin treatment as an uninformative covariate (Arnold 2010) and did not consider this model further.

Our analysis indicated that male ferrets supported significantly larger infestations of *O. hirsuta* (X^2=7.45, P=0.006). Males had almost three times the average number of *O. hirsuta* than females (males $\bar{x}$=8.64±14.04, females $\bar{x}$=3.03±4.97). Year also had a significant influence on the number of *O. hirsuta* recovered from ferrets, where ferrets examined in 2010 had a lower mean abundance of *O. hirsuta* than in 2009, 2011, and 2012 (X^2=17.32, P< 0.001). The mean number of *O. hirsuta* collected from ferrets ranged from 2.37 to 11.62 from 2009 to 2011 (2009:

TABLE 5. Ten a priori negative-binomial distributed generalized linear models predicting abundance of the flea *Oropsylla hirsute* on black-footed ferrets (*Mustela nigripes*), number of variables (k), model residual sum of squares (RSS), corrected Akaike's Information Criterion (AIC_c), ΔAIC_c, and Akaike weight (w_i).

Model	k	RSS	AIC_c	ΔAIC_c	w_i
sex,year	4	76.88	379.86	0	0.44
sex,year,treat	5	76.76	380.02	0.16	0.41
sex,age,year,day,treat	7	76.56	383.72	3.86	0.06
sex,age,year,day	6	76.80	384.19	4.33	0.05
sex,age,year,day,sex*age,treat	8	76.58	385.90	6.04	0.02
sex,age,year,day,sex*age	7	76.91	386.43	6.57	0.02
intercept only	2	76.11	391.39	11.53	0.00
sex,age,year,day,colony	7	74.89	406.54	26.68	0.00
sex,age,year,day,treat,colony	8	74.86	410.20	30.34	0.00
sex,age,year,day,treat,colony,treat*colony	9	74.86	410.20	30.34	0.00

$\bar{x}$=4.96±8.10; 2010: $\bar{x}$=2.37±4.66; 2011: $\bar{x}$=11.62±16.39; and 2012: $\bar{x}$=11.57±17.06). In our study, treatment with deltamethrin did not influence the abundance of *O. hirsuta* on captured ferrets (X^2=2.3, P=0.13). Abundance of fleas from ferrets captured on treated colonies ($\bar{x}$=5.0±11.17) were similar to those captured on untreated colonies ($\bar{x}$=6.14±10.8). Additionally, there were no significant differences in the abundance of *O. hirsuta* between juvenile and adult ferrets, standardized Julian date, or colony of capture.

DISCUSSION

In our study, *O. hirsuta* was the primary flea species associated with ferrets and some fleas collected from ferrets carried *Y. pestis*. The *O. hirsuta* is one of the primary fleas of prairie dogs (Salkeld and Stapp 2008; Jones and Britten 2010; Mize and Britten 2016) and has been previously reported as a common parasite of ferrets (Harris et al. 2014). The flea species collected from these ferrets strongly resembles flea faunas found on black-tailed prairie dogs (96.8%; 447/462; *O. hirsuta*) and in their burrows (75.7%; 240/317; *O. hirsuta*) at our study site (Britten and Mize 2010). *Oropsylla hirsuta* is known to carry *Y. pestis* and has a significant role in plague epizootic dynamics (Wilder et al. 2008a, b).

Black-footed ferrets experienced a severe population bottleneck, a process often associated with a loss of parasite diversity (Gompper and Williams 1998). There is a paucity of information in the historic record about parasites specific to ferrets before their population decline in the wild (Gompper and Williams 1998). *Rhadinopsylla fraterna* was collected from a ferret recorded near Jordan, Montana, in 1937; this flea species was also recorded from a suite of rodent hosts as well as long-tailed weasels (*Mustela frenata*, Holland 1985). *Oropsylla hirsuta* has also been collected from ferrets in Powder River County, Montana (Hubbard 1947) and near Chamberlain, South Dakota (Boddicker 1968). More recently, reintroduced ferrets captured in the Conata Basin, South Dakota, yielded *O. hirsuta*, *O. tuberculata*, and *Pulex irritans* as well as *E. wenmanni* and *Peromyscopsylla hesperomys* (Harris et al. 2014). Here we report the first collection of *Peromyscopsylla selenis* from a ferret, a flea documented to be associated with a variety of small rodents (Hubbard 1947).

In our study, male ferrets had a higher abundance of fleas than female ferrets. Morphological, physiological, and behavioral characteristics might explain why males harbor more fleas than females; though this difference is not unique to ferrets (Brinkerhoff et al. 2006; Krasnov 2008; Lopez et al. 2013). Male ferrets are almost twice as large as females (Anderson et al. 1986), thus males have more surface area to support a higher population of ectoparasites. Although males are usually less than half as abundant as females within a typical ferret population (Forrest et al. 1988), the home ranges of male ferrets are about twice as large as the home ranges of female ferrets (Jachowski et al. 2010; Eads et al. 2011; Livieri and Anderson 2012) and typically overlap several female ferrets' ranges (Powell 1979; Livieri and Anderson 2012). This pattern of space use provides males a high potential to receive fleas from prairie dogs, burrows, and other ferrets, over a relatively large area. Variation in infestation based on host sex also may be due in part to differences in host defenses and physiology, flea phenology, or environmental factors (Krasnov 2008; Krasnov et al. 2012; Kiffner et al. 2013). While all *Y. pestis*–positive fleas collected were from male ferrets, our power to detect significant differences in the number of *Y. pestis*–positive fleas between male and female ferrets was insufficient.

Our models indicated there was a difference in *O. hirsuta* abundance between years. Variation between years could be due to annual variation in weather such as temperature and precipitation. Fleas are sensitive to changes in microclimate of their hosts' nests or burrows such as temperature and relative humidity changes, which can alter egg production, development time, and survival rates (Marshall 1981; Krasnov 2008). In addition, fleas are also sensitive to larger scale weather conditions; Stenseth et al. (2006) suggested warmer spring temperatures and higher summer relative humidity increased flea burdens on great gerbils (*Rhombomys opimus*) in Kazakhstan. However, Eads et al. (2016) observed the highest flea densities on black-tailed prairie dogs during the driest year of a 3 yr study in New Mexico.

Contrary to our expectations, treatment of colonies with deltamethrin did not influence the abundance of fleas collected from captured ferrets. Deltamethrin is a water-insoluble insecticide that is commonly used to control fleas and prevent deaths of prairie dogs and ferrets from plague (Biggins et al. 2010; Matchett et al. 2010). Several factors might explain this observation. During this study, not all colonies were treated with deltamethrin. Ferrets that were captured on treated colonies could have been born and reared on untreated colonies but captured during dispersal on treated colonies carrying fleas from their natal, untreated colonies. The effectiveness of the insecticide might have been diminished by the length of time between treatment and ferret capture on specific colonies, excessive precipitation, or a combination of both. Another possible explanation might include human error during the deltamethrin application process: an inadequate amount of insecticide could have been applied during treatment. This finding reflects the difficulty in determining the spatial scale at which deltamethrin should be applied and the efficacy of treating relatively small colonies that comprise a relatively small proportion of the total landscape; targeting small islands for treatment may not effectively reduce flea infestations when the surroundings may be an ocean of plague.

While we did not examine individual hosts for exposure to *Y. pestis*, we did provide evidence that ferrets carried *Y. pestis*–positive fleas before major plague epizootics occurred in prairie dog populations in 2011 and 2013. Prevalence of *Y. pestis*–positive fleas collected from ferrets at Lower Brule ranged from 0% to 2.8% during our study, which was similar to *Y. pestis* prevalence of 3% in fleas collected from prairie dog burrows in 2009 and 2010 (Britten and Mize 2010).

To the best of our knowledge, there has been only one other study to examine prevalence of *Y. pestis* in fleas collected from ferrets. Mouse inoculation and single-reaction PCR tests failed to detect *Y. pestis* in about 700 fleas collected from ferrets (n=70) between 1996 and 2005 at the U. L. Bend National Wildlife Refuge (NWR), Montana (Matchett et al. 2010). However, in 2006–07, *Y. pestis* was detected in fleas (n=373) collected from two male and one female ferrets with a prevalence of ferrets infested with *Y. pestis*–positive fleas of 9.4% (3/32) at U. L. Bend NWR using the same nested PCR protocol as our study (Matchett et al. 2010. Failure to detect *Y. pestis* in fleas collected from ferrets between 1996 and 2005 at U. L. Bend NWR may be due, in part, to a difference in the methods used to detect *Y. pestis*, because the nested PCR assay is more sensitive than mouse inoculation or single-reaction PCR assays (Hanson et al. 2007; Matchett et al. 2010).

Our data provided evidence that ferrets are capable of transporting *Y. pestis*–infected fleas within and among prairie dog colonies. It appears plausible that vaccinated ferrets could contribute to the spread of *Y. pestis* by promoting connections among prairie dog coteries in a similar manner as has been reported for northern grasshopper mice (Stapp et al. 2009; Salkeld et al. 2010; Kraft and Stapp 2013) and by promoting connections among colonies. Ferrets, whose territories can range between 56 and 137 ha (Jachowski et al. 2010; Fagerstone and Biggins 2011; Livieri and Anderson 2012), would be expected to move frequently among coteries of black-tailed prairie dogs that range in size from 0.05 to 1.01 ha (Hoogland 1995). Additionally, Richardson et al. (1987) reported that during the winter months, nightly movements of ferrets averaged 1406 m, were nonlinear, and increased during the breeding season, and Biggins et al. (2006) reported that ferrets are capable of traversing their entire home ranges in 12 hr. Male ferrets may be particularly important in the movement of fleas and *Y. pestis* across the landscape because males have larger home ranges than females (Jachowski et al. 2010; Eads et al. 2011; Livieri and Anderson 2012) and males disperse farther and almost always leave their natal areas (Clark et al. 1986; Forrest et al. 1988), whereas females usually disperse short distances, if at all (Biggins et al. 1986; Forrest et al. 1988). These space use patterns are noteworthy because, as we have reported here, male ferrets harbor larger infestations of fleas.

Movements among prairie dog colonies by ferrets are not uncommon (Biggins et al. 1985; Richardson et al. 1987; Forrest et al. 1988) but would be much less frequent than movements among coteries. Intercolony movements would likely be influenced by colony size, distance between colonies, season, sex, and perhaps intra- and interspecific competition. At our study site, intercolony movements most often occurred by juveniles during dispersal (September–November; Forrest et al. 1988) and adult males during the breeding season (March–April). During epizootics, as areas of each affected colony succumb to plague and prey disappears, ferrets might move to other areas of the colony or to other colonies seeking prey, potentially carrying fleas and *Y. pestis* with them and infecting additional areas, exacerbating disease transmission.

Because of the rarity of ferrets, they are not likely to be significant agents in dispersing *Y. pestis* on a large scale. However, given their close association with prairie dogs, overlapping flea faunas, and movements within and between colonies, ferrets might have an incidental but potentially important role in transporting infected fleas, possibly leading to plague epizootics on local scales.

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LITERATURE CITED

Anderson E, Forrest SC, Clark TW, Richardson L. 1986. Paleobiology, biogeography, and systematics of the black-footed ferret, *Mustela nigripes* (Audubon and Bachman), 1851. *Great Basin Nat Mem* 8:11–62.

Arnold TW. 2010. Uninformative parameters and model selection using Akaike's information criterion. *J Wildl Manage* 74:1175–1178.

Biggins DE, Godbey JL, Gage KL, Carter LG, Montenieri JA. 2010. Vector control improves survival of three species of prairie dogs (*Cynomys*) in areas considered enzootic for plague. *Vector-Borne Zoonot Dis* 10:17–26.

Biggins DE, Godbey JL, Matchett MR, Hanebury LR, Livierie TM, Marinari PM. 2006. Monitoring black-footed ferrets during reestablishment of free-ranging populations: Discussion of alternative methods and recommended minimum standards. In: Roelle JE, Miller BJ, Godbey JL, Biggins DE, editors. *Recovery of the black-footed ferret: Progress and continuing challenges*. US United States Geological Survey Scientific Investigations Report 2005-5293. Reston, Virginia, 155–174.

Biggins DE, Schroeder MH, Forrest SC, Richardson L. 1985. Movements and habitat relationships of radio-tagged black-footed ferrets. In: *Proceedings of the black-footed ferret workshop,* Anderson SH and Inkley, editors. Cheyenne, Wyoming, 18–19 September 1984. US Wyoming Game and Fish Department, pp. 11.1–11.17.

Biggins DE, Schroeder MH, Forrest SC, Richardson L. 1986. Activity of radio-tagged black-footed ferrets. *Great Basin Nat Mem* 8:135–140.

Boddicker M. 1968. Parasites of the black-footed ferret. *Proc S D Acad Sc*i 47: 141–148.

Brinkerhoff RJ. 2008. *Mammal and flea occurrence in association with black-tailed prairie dog* (Cynomys ludovicianus) *colonies: Implications for interspecific plague transmission*. PhD Thesis, Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, Colorado, 176 pp.

Brinkerhoff RJ, Collinge SK, Bai Y, Ray C. 2009. Are Carnivores universally good sentinels of plague? *Vector-Borne Zoonot* 9:491–497.

Brinkerhoff RJ, Markeson AB, Knouft JH, Gage KL, Monteneri JA. 2006. Abundance patterns of two O*ropsylla* (Ceratophyllidae: Siphonaptera) species on black-tailed prairie dog (*Cynomys ludovicianus*) hosts. *J Vector Ecol* 31:355–363.

Brinkerhoff RJ, Martin AP, Jones RT, Collinge SK. 2011. Population genetic structure of the prairie dog flea and plague vector, *Oropsylla hirsuta*. *Parasitology* 138:71–79.

Britten HB, Mize EL. 2010. Plague prevalence in Lower Brule Indian Reservation black-tailed prairie dog colonies. *Fish, and Parks Report*. South Dakota Department of Game, Pierre, South Dakota.

Burnham KP, Anderson DR. 2004. Multimodel inference: Understanding AIC and BIC in Model Selection. *Soc Meth Res* 33:261–304.

Clark TW, Richardson L, Forrest SC, Casey DE, Campbell TM III. 1986. Descriptive ethology and activity patterns of black-footed ferrets. *Great Basin Nat Mem* 8:115–134.

Eads DA, Biggins DE, Doherty PF Jr., Gage KL, et al. 2013. Using occupancy models to investigate the prevalence of ectoparasitic vectors on hosts: An example with fleas on prairie dogs. *Int J Parasitol* 2: 246–256.

Eads DA, Biggins DE, Livieri TM, Millspaugh JJ. 2014. Space use, resource selection and territoriality of black-footed ferrets: Implications for reserve design. *Wildlife Biol* 20:27–36.

Eads DA, Biggins DE, Long DH, Gage KL, et al. 2016. Droughts may increase susceptibility of prairie dogs to fleas: Incongruity with hypothesized mechanisms of plague cycles in rodents. *J Mammal* 97:1044–1053.

Eads DA, Millspaugh JJ, Biggins DE, Livieri TM, et al. 2011. Postbreeding resource selection by adult black-footed ferrets in the Conata Basin, South Dakota. *J Mammal* 92:760–770.

Fagerstone KA, Biggins DE. 2011. Black-footed ferret areas of activity during late summer and fall at Meeteetse, Wyoming. *J Mammal* 92:705–709.

Forrest SC, Biggins DE, Richardson L, Clark TW, Campbell TM III. 1988. Population attributes for the black-footed ferret *Mustela nigripes* at Meeteetse, Wyoming, 1981–1985. *J Mammal* 69:261–273.

Furman DP, Catts EP. 1982. Order Siphonaptera. In: Furman DP, Catts EP, editors. *Manual of Medical Entomology,* 4th ed. Cambridge University Press, New York, New York, pp. 138–157.

Gage KL. 1999. Plague Surviellance. In: Dennis DT, Gage KL, Gratz NG, Poland JD, Tikhomirov E, editors. *Plague manual: Epidemiology, distribution, surveillance and control*. Epidemic Disease Control, World Health Organization, Geneva, Switzerland, pp. 135–166.

Gage KL, Kosoy MY. 2005. Natural history of plague: Perspectives from more than a century of research. *Annu Rev Entomol* 50:505–528.

Gage KL, Kosoy MY. 2006. Recent trends in plague ecology. In: Roelle JE, Miller BJ, Godbey JL, Biggins DE, editors. *Recovery of the black-footed ferret: Progress and continuing challenges*. United States Geological Survey Scientific Investigations Report 2005-5293. Reston, Virginia, 213–231.

Godbey JL, Biggins DE, Garelle D. 2006. Exposure of captive black-footed ferrets to plague and implications for species recovery. In: Roelle JE, Miller BJ, Godbey JL, Biggins DE, editors. *Recovery of the black-footed ferret: Progress and continuing challenges*. United States Geological Survey Scientific Investigations Report 2005-5293. Reston, Virginia, 233–237.

Gompper ME, Williams ES. 1998. Parasite conservation and the black-footed ferret recovery program. *Conserv Biol* 12:730–732.

Hanson DA, Britten HB, Restani M, Washburn LR. 2007. High prevalence of *Yersinia pestis* in black-tailed prairie dog colonies during an apparent enzootic phase of sylvatic plague. *Conserv Genet* 8: 789–795.

Harris NC, Livieri TM, Dunn RR. 2014. Ectoparasites in black-footed ferrets (*Mustela nigripes*) from the largest reintroduced population of the Conata Basin, South Dakota, USA. *J Wildlife Dis* 50:340–343.

Hinnebusch J, Schwan T. 1993. New method for plague surveillance using polymerase chain reaction to detect *Yersinia pestis* in fleas. *J Clin Microbiol* 31: 1511–1514.

Holland GP. 1985. The fleas of Canada, Alaska and Greenland (Siphonaptera). *Mem Entomol Soc Can* 117:3–632.

Hoogland JL. 1995. *The black-tailed prairie dog: Social life of a burrowing mammal*. University of Chicago Press. Chicago, Illinois, 557 pp.

Hubbard CA, 1947. *Fleas of western North America*. Iowa State College Press, Ames, Iowa, 533 pp.

Jachowski DS, Millspaugh JJ, Biggins DE, Livieri TM, Matchett MR. 2010. Home-range size and spatial organization of black-footed ferrets *Mustela nigripes* in South Dakota, USA. *Wildl Biol* 16:66–76.

Jones PH, Britten HB. 2010. The absence of concordant population genetic structure in the black-tailed prairie dog and the flea, *Oropsylla hirsuta,* with implications for the spread of *Yersinia pestis*. *Mol Ecol* 19:2038–2049.

Kiffner C, Stanko M, Morand S, Khokhlova IS, Shenbrot GI, Laudisoit A, Leirs H, Hawlena H, Krasnov BR. 2013. Sex-biased parasitism is not universal: Evidence from rodent-flea associations from three biomes. *Oecologia* 173:1009–1022.

Kraft JP, Stapp P. 2013. Movements and burrow use by northern grasshopper mice as a possible mechanism of plague spread in prairie dog colonies. *J Mammal* 94:1087–1093.

Krasnov BR. 2008. *Functional and evolutionary ecology of fleas: A model for ecological parasitology*. Cambridge University Press, New York, New York, 593 pp.

Krasnov BR, Bordes F, Khokhlova IS, Morand S. 2012. Gender-biased parasitism in small mammals: Patterns, mechanisms, consequences. *Mammalia* 76:1–13.

Lewis RE. 2002. A review of the North American species of *Oropsylla* Wagner and Ioff, 1926 (Siphonaptera: Ceratophyllidae: Ceratophyllinae). *J Vector Ecol* 27: 184–206.

Livieri TM, Anderson EM. 2012. Black-footed ferret home ranges in Conata Basin, South Dakota. *West N Am Naturalist* 72:196–205.

Lopez J, Wey TW, Blumstein DT. 2013. Patterns of parasite prevalence and individual infection in yellow-bellied marmots. *J Zool* 291:296–303.

Margolis L, Esch GW, Holmes JC, Kuris AM, Schad GA. 1982. The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). *J Parasitol* 68:131–133.

Marshall AG. 1981. *The ecology of ectoparasitic insects*. Academic Press, New York, New York.

Matchett MR, Biggins DE, Carlson V, Powell B, Rocke T. 2010. Enzootic plague reduces black-footed ferret (*Mustela nigripes*) survival in Montana. *Vector-Borne Zoonot Dis* 10:27–35.

Mize EL, Britten HB. 2016. Detections of *Yersinia pestis* east of the known distribution of active plague in the United States. *Vector-Borne Zoonot Dis* 16:88–95.

Powell BS, Andrews GP, Enama JT, Jendrek S, Bolt C, Worsham P, Pullen JK, Wilson R, Hines H, Smith L, et al. 2005. Design and testing for a nontagged F1-V fusion protein as vaccine antigen against bubonic and pneumonic plague. *Biotechnol Progr* 21:1490–1510.

Powell RA. 1979. Mustelid spacing patterns: Variations on a theme by *Mustela*. *Z Tierpsychol* 50:153–165.

R Development Core Team. 2013. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. Vienna, Austria. http://www.R-project.org. Accessed January 2017.

Richardson L, Clark TW, Forrest SC, Campbell TM III. 1987. Winter ecology of black-footed ferrets (*Mustela nigripes*) at Meeteetse, Wyoming. *Amer Midl Nat* 117:225–239.

Rocke TE, Mecher J, Smith SR, Friedlander AM, Andrews GP, Baeten LA. 2004. Recombinant F1-V fusion protein protects black-footed ferrets (*Mustela nigripes*) against virulent *Yersinia pestis* infection. *J Zoo Wildl Med* 35:142–146.

Rocke TE, Smith S, Marinari P, Kreeger J, Enama JT, Powell BS. 2008. Vaccination with F1-V fusion protein protects black-footed ferrets (*Mustela nigripes*) against plague upon oral challenge with *Yersinia pestis*. *J Wildl Dis* 44:1–7.

Rózsa L, Reiczigel J, Majoros G. 2000. Quantifying parasites in samples of hosts. *J Parasitol* 86:228–232.

Salkeld DJ, Eisen RJ, Stapp P, Wilder AP, Lowell J, Tripp DW, Alberson D, Antolin MF. 2007. The potential role of swift foxes (*Vulpes velox*) and their fleas in plague outbreaks in prairie dogs. *J Wildl Dis* 43:425–431.

Salkeld DJ, Salathé M, Stapp P, Jones JH. 2010. Plague outbreaks in prairie dog populations explained by percolation thresholds of alternate host abundance. *Proc Natl Acad Sci U S A* 107:14247–14250.

Salkeld DJ, Stapp P. 2006. Seroprevalence rates and transmission of plague (*Yersinia pestis*) in mammalian carnivores. *Vector-Borne Zoonot Dis* 6:231–239.

Salkeld DJ, Stapp P. 2008. Prevalence and abundance of fleas in black-tailed prairie dog burrows: Implications for the transmission of plague (*Yersinia pestis*). *J Parasitol* 94:616–621.

Salkeld DJ, Stapp P. 2009. Effects of weather and plague-induced die-offs of prairie dogs on the fleas of northern grasshopper mice. *J Med Entomol* 46:588–594.

Santymire RM, Wisely SM, Livieri TM, Howard J. 2012. Using canine width to determine age in the black-footed ferret *Mustela nigripes*. *Small Carnivore Conserv* 46:17–21.

Seery DB, Biggins DE, Montenieri JA, Enscore RE, Tanda DT, Gage KL. 2003. Treatment of black-tailed prairie dog burrows with deltamethrin to control fleas (Insecta: Siphonaptera) and plague. *J Med Entomol* 40:718–722.

Shaw DJ, Grenfell BT, Dobson AP. 1998. Patterns of macroparasite aggregation in wildlife host populations. *Parasitology* 117:597–610.

Sodeinde OA, Goguen JD. 1988. Genetic analysis of the 9.5-kilobase virulence plasmid of *Yersinia pestis*. *Infect Immun* 56:2743–2748.

Stapp P, Salkeld DJ, Franklin HA, Kraft JP, Tripp DW, Antolin MF, Gage KL. 2009. Evidence for the involvement of an alternate rodent host in the dynamics of introduced plague in prairie dogs. *J Anim Ecol* 78:807–817.

Stenseth NC, Samia NI, Viljugrein H, Kausrud KL, Begon M, Davis S, Leirs H, Dubyanskiy VM, Esper J, Ageyev VS, et al. 2006. Plague dynamics are driven by climate variation. *Proc Nat Acad Sci U S A* 103:13110–13115.

Thorne ET, Williams ES. 1988. Disease and endangered species: The black-footed ferret as a recent example. *Conserv Biol* 2:66–74.

US Fish and Wildlife Service (USFWS). 1967. Native fish and wildlife endangered species. *Federal Register* 32:4001.

US Fish and Wildlife Service (USFWS). 2012. Endangered and threatened wildlife and plants: Revised recovery plan for the black-footed ferret. *Federal Register* 78:77485–77486.

Venables WN, Ripley BD. 2002. *Modern applied statistics with S*. Springer, New York, New York, 495 pp.

Wilder AP, Eisen RJ, Bearden SW, Montenieri JA, Gage KL, Antolin MF. 2008a. *Oropsylla hirsuta* (Siphonaptera: Ceratophyllidae) can support plague epizootics in black-tailed prairie dogs (*Cynomys ludovicianus*) by early-phase transmission of *Yersinia pestis*. *Vector-Borne Zoonot Dis* 8:359–368.

Wilder AP, Eisen RJ, Bearden SW, Montenieri JA, Tripp DW, Brinkerhoff RJ, Gage KL, Antolin MF. 2008b. Transmission efficiency of two flea species (*Oropsylla tuberculata cynomuris* and O*ropsylla hirsuta*) involved in plague epizootics among prairie dogs. *EcoHealth* 5:205.

Wilson K, Bjørnstad ON, Dobson AP, Merler S, Poglayen G, Randolph SE, Read AF, Skorping A. 2002. Heterogeneities in macroparasite infections: Patterns and processes. In: Hudson PJ, Rizzoli A, Grenfell B, Heesterbeek H, Dobson AP, editors. *Ecology of* *wildlife disease*. Oxford University Press, New York, New York, pp. 6–44.

Zuur A, Ieno EN, Walker NJ, Saveliev AA, Smith GM. 2009. GLM and GAM for count data. In: Zuur A, Ieno EN, Walker NJ, Saveliev AA, Smith GM editors. *Mixed effects models and extensions in ecology with R*. Springer, New York, New York, pp. 209–244.

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